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AN EXPERIMENTAL STUDY OF ANTAGONISTIC EFFECTS OF HOMOEOPATHIC MEDICINES AGAINST *PSEUDOMONAS AERUGINOSA*

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ABSTRACT *Pseudomonas aeruginosa* is one of the major bacteria for nosocomial infections because the conditions are favourable for its growth and with multidrug resistance to various antibiotics. Homoeopathy, the system of medicines which doesn't show any adverse reaction is considered safe for administration. The homoeopathic medicines Barosma Crenata 30CH, Holarrhena Antidysenterica 30CH, Laurocerasus 6CH gave more than 75% inhibition in single-dose, whereas gave inhibition of more than 84% when administered in repetition. These medicines were also given in conjunction with different medicines in double and triple doses where inhibition was more than 83% and 87% respectively. Out of all the medicines selected for the experiments, Laurocerasus 6CH gave the most inhibition i.e. 90.91%, which gave more inhibition then Doripenem per cent inhibition (86%).

KEYWORDS : *Pseudomonas aeruginosa*, Multi-drug resistance, Posology, Homoeopathy.

INTRODUCTION

Pseudomonas aeruginosa is one of the major cause of infections caused in hospitals and lies third among organisms causing nosocomial infections[1][2]. As the environmental conditions are favourable for its growth, the infection caused is rapid and life-threatening[3]. The symptoms produced are fever with chills, pneumonia, abscess, otitis media, infection of burns, urinary tract infection[4], etc. The major reason for the increase in infection by *Pseudomonas aeruginosa* is its cultural characteristics[5] of growing aerobically at room temperature at wide range 6-42℃ with rapid development resistance to anti-bacterial drugs[6]. Resistant bacteria are not killed, or which even grow or multiply under the influence of antibiotic. As bacteria turn out to be antibiotic-resistant[7], it can result in serious illness and infections to humans.

Doripenem[8] is a broad-spectrum antibiotic from class Carbapenem. It is effective antibacterial drug against community-acquired infections (pneumonia, bronchitis, UTI, gastroenteritis), which are caused by gram-negative bacteria[9]. Doripenem kills the bacteria by preventing them from forming the cell wall.

An experimental study is a basis for understanding the role of any drug against a specific organism. Homoeopathic medicines have been proved to be effective against a lot of organisms; though in-vitro[10][11] experiments are less documented. Hence an experimental study was undertaken to know the effects of various preparations of Holarrhena antidysenterica[12], Barosma crenata[13] and Laurocerasus[14] on *Pseudomonas aeruginosa*. The medicines were selected based on rubric *Pseudomonas aeruginosa*[15] produced by an infection caused by the bacterium.

Holarrhena antidysenterica[16] from family *Apocynaceae*, is commonly known as Kurchi. The mother tincture is prepared from dried bark of the plant and it grows throughout India. It has a very good action on acute and chronic dysentery. *Barosma crenata*[17] of family *Rutaceae*, native plant from South Africa. The mother tincture is prepared from the leaf of this plant. It is effective for the genito-urinary system, mucopurulent discharges, and irritable bladder with cystitis, prostatic disorders and leucorrhea. *Laurocerasus*[18] of family *Rosaceae,* common name Cherry laurel is found in Persia & Turkey and is cultivated in temperate regions. The part used for the preparation of mother tincture is the leaf. Clinically it is used in cardio-respiratory disorders like cyanosis neonatrum, mitral regurgitation, paralysis of lungs, etc.

The medicines selected were prepared according to Decimal and Centesimal scales. Decimal scale[19] was introduced by Dr Constantine Hering, with drug vehicle ratio 1:9 and drug strength of 1/10 based on the principle that the first potency has 1/10th part of the mother tincture; and the next potency has 1/10th part of the previous potency. The decimal scale is denoted by 'X' i.e. potency is written as 1X, 2X and so on. Centesimal scale[20] was introduced by Dr Samuel Hahnemann. He mentioned this scale in 5th edition of Organon of Medicine under aphorism 270. The designation used is 'C' or 'CH'. The drug vehicle ratio for centesimal scale is 1:99 and the drug strength is based on the principle that first potency should contain 1/100th part of the mother tincture and the next potency should have 1/100th part of the previous potency.

The aim for experimenting was to know that the above-mentioned medicines show any anti-microbial activity against the bacterium and in which potency.

MATERIALS AND METHODS

Organism:

The bacterium *Pseudomonas aeruginosa* (Accession No. - MCC 2080) isolated from blood and under risk group II was procured from the National Centre for Microbial Resource, National Centre for Cell Science, Pune, India.

MEDIA AND CHEMICALS:

All the chemicals and media were purchased from Hi-Media

Laboratories, Mumbai, India. Doripenem was purchased from GMA Media Pvt.ltd. India.

Homoeopathic Medicines:

The homoeopathic medicines

- *Holarrhena antidysenterica* were selected in following potencies -
- 3X, 6CH, 12CH, 30CH, 200CH, 1M
- *Barosma crenata* were selected in following potencies -
- 3X, 6CH, 12CH, 30CH, 200CH, 1M
- *Laurocerasus* were selected in following potencies -
- 3X, 6CH, 12CH, 30CH, 200CH, 1M

Were made available from St. George Homoeopathy pharmaceuticals, India a GMP certified pharmaceuticals.

96 Microwell plate assay:

- **Single-dose administration –**

For observing the antimicrobial action of selected medicines, 96 microwell plates (Tarsons Pvt. Ltd. Kolkata, India.) were used for experiments. The content of the wells was 130μl of homoeopathic medicines, 110μl of media and 10μl of culture that sum up about 250μl in total.

The wells with controls consist of-

- Positive control - Selected standard antibiotic Doripenem-130μl with MH Broth-110μl, and Culture-10μl.
- Vehicle control - Dispensing alcohol (90%) -130μl with MH Broth-110μl and Culture-10μl.
- Negative control - MH Broth 240μl with culture 10μl.
- Media control - MH Broth 250μl

The plates were kept in an incubator at 37℃ for 24 hours and were observed in Spectrophotometer (Epoch, BioTek Instruments) for optical density at 600nm.

- **Double dose administration –**

The potencies of selected homoeopathic medicines showing maximum per cent inhibition against the bacterium were used for further experiments. The experiments were customized to administer homoeopathic medicines in two doses of 65μl per dose with an interval of 8 hours. Vehicle control (Dispensing alcohol 90%) was also poured in two doses of 65μl per dose. The controls used were the same as mentioned above. The plates were incubated for 24 hours at 37℃.

- Double dose administration of different homoeopathic medicines –

The above homoeopathic medicines were used in conjunction in this experiment. The experiment had two doses of medicines administered at 8 hours of intervals of 65μl each dose. The vehicle was also poured in the same manner as of homoeopathic medicines i.e. in two doses of 65μl per dose. The doses were –

I. *Barosma crenata* 30CH followed by *Holarrhena antidysenterica* 30CH.
ii. *Holarrhena antidysenterica* 30CH followed by *Laurocerasus* 6CH.
iii. *Barosma crenata* 30CH followed by *Laurocerasus* 6CH.

- Triple dose administration of different homoeopathic medicines –As per the previous experiment the same medicines were administered in triple doses of 45μl per dose with an interval of 4 hours. The doses were –

i. *Barosma crenata* 30CH - *Barosma crenata* 30CH - *Holarrhena antidysenterica* 30CH.
ii. *Barosma crenata* 30CH - *Barosma crenata* 30CH - *Laurocerasus* 6CH.
iii. *Holarrhena antidysenterica* 30CH - *Holarrhena antidysenterica* 30CH - *Barosma crenata* 30CH
iv. *Holarrhena antidysenterica* 30CH - *Holarrhena antidysenterica* 30CH - *Laurocerasus* 6CH.
v. *Laurocerasus* 6CH - *Laurocerasus* 6CH - *Barosma crenata* 30CH.
vi. *Laurocerasus* 6CH - *Laurocerasus* 6CH - *Holarrhena antidysenterica* 30CH.
vii. *Barosma crenata* 30CH - *Holarrhena antidysenterica* 30CH - *Laurocerasus* 6CH.

The vehicle (dispensing alcohol 90%) was also poured in 3 doses of 45μl each while the rest of the control was the same. The microwell plates were kept for incubation for 24 hours at 37℃.

Statistical Analysis:

The experiments were conducted in triplicates. The mean of the readings with standard deviation was calculated with the help of Graph-Pad Prism software, Version 8.1. The One-way ANOVA test was used for determining the statistical significance of p-value ≤0.001. The per cent inhibition was calculated by counting vehicle control as 100%.

RESULTS

Per cent inhibition:

Table 1 represents the optical density of *Pseudomonas aeruginosa* under the influence of homoeopathic medicines. The homoeopathic medicines are grouped according to their increasing potencies. Data includes optical density mean of triplicate readings with their standard deviation (One way ANOVA test, Graph-Pad Prism Software) and inhibition percentage with p-value is 0.0001, N=3.

TABLE 1: ANTIBACTERIAL ACTIVITY OF HOMOEOPATHIC MEDICINES IN A SINGLE DOSE

HOMOEOPATHIC MEDICINES	POTENCY	OPTICAL DENSITY AGAINST *P. aeruginosa* (Mean ± Standard deviation) in nm.	PER CENT (%)INHIBITION
Barosma crenata	3X	0.361 ± 0.03	75.70
	6CH	0.378 ± 0.02	74.48
	12CH	0.351 ± 0.04	76.37
	30 CH	0.340 ± 0.04	77.04
	200 CH	0.358 ± 0.03	75.83
	1M	0.341 ± 0.06	76.04
Holarrhena antidysenterica	3X	0.353 ± 0.044	76.64
	6CH	0.439 ± 0.039	70.98
	12CH	0.334 ± 0.007	76.10
	30 CH	0.326 ± 0.024	77.72
	200 CH	0.352 ± 0.023	77.45
	1M	0.336 ± 0.018	73.68
Laurocerasus	3X	0.347 ± 0.019	76.23
	6CH	0.331 ± 0.049	77.98
	12CH	0.355 ± 0.048	77.51
	30 CH	0.431 ± 0.040	70.44
	200 CH	0.335 ± 0.020	76.30
	1M	0.391 ± 0.066	77.31
Doripenem		0.143 ±0.008	90.78

The homoeopathic medicines *Barosma crenata* 30CH, *Holarrhena antidysenterica* 30CH, *Laurocerasus* 6CH showed more than 70% inhibition. For further experiments, these homoeopathic medicines were poured twice of 65μl each dose of respective potencies. The experimental data contains mean of triplicate readings and standard deviation where inhibition percentage recorded was 84.10%, 85.36% and 84.03% respectively (Table 2).

TABLE 2: ANTIBACTERIAL ACTIVITY OF HOMOEOPATHIC MEDICINES IN DOUBLE DOSE.

HOMOEOPATHIC MEDICINES	POTENCY	OPTICAL DENSITY AGAINST *P. aeruginosa* (Mean ± Standard deviation) in nm.	PER CENT (%)INHIBITION

Barosma crenata	30CH	0.240 ± 0.011	84.10
Holarrhena antidysenterica	30CH	0.221 ± 0.019	85.36
Laurocerasus	6CH	0.241 ± 0.006	84.03
Doripenem		0.143 ± 0.025	90.78

The above medicines were used in conjunction and were poured in two doses of 65µl per dose (Table 3), the inhibition percentage of *Barosma crenata* 30CH, *Holarrhena antidysenterica* 30CH, *Lauroceraus* 6CH in conjunction was more than 83%. The mean and standard deviation of the experiment were calculated from triplicate readings.

TABLE 3: ANTIBACTERIAL ACTIVITY OF DIFFERENT HOMOEOPATHIC MEDICINES IN DOUBLE DOSE.

HOMOEOPATHIC MEDICINES	OPTICAL DENSITY AGAINST P. aeruginosa (Mean ± Standard deviation) in 600nm.	PER CENT (%)INHIBITION
Barosma crenata 30CH + *Holarrhena antidysenterica* 30CH	0.245±0.034	83.60
Holarrhena antidysenterica 30CH + *Laurocerasus* 6CH	0.212±0.003	85.81
Barosma crenata 30CH + *Laurocerasus* 6CH	0.247±0.015	83.46
Doripenem	0.210±0.008	88.31

The same medicines were poured in three doses of 45µl per dose. The experiment was repeated thrice and mean and standard deviation were calculated with proper statistics where inhibition percentage was more than 87%, with chemical control (Doripenem) was 86.72% (Table 4) (p-value 0.0001, N=3)

TABLE 4: ANTIBACTERIAL ACTIVITY OF DIFFERENT HOMOEOPATHIC MEDICINES IN TRIPLE DOSE.

HOMOEOPATHIC MEDICINES	OPTICAL DENSITY AGAINST P. aeruginosa (Mean ± Standard deviation) in 600nm.	PER CENT (%) INHIBITION
Barosma crenata 30CH + *Barosma crenata* 30CH *Holarrhena antidysenterica* 30CH	0.189±0.023	87.46
Barosma crenata 30CH + *Barosma crenata* 30CH + *Laurocerasus* 6CH	0.178±0.014	88.19
Holarrhena antidysenterica 30CH + *Holarrhena antidysenterica* 30CH + *Barosma crenata* 30CH	0.185±0.013	87.73
Holarrhena antidysenterica 30CH + *Holarrhena antidysenterica* 30CH + *Laurocerasus* 6CH	0.165±0.032	89.06
Laurocerasus 6CH + *Laurocerasus* 6CH + *Barosma crenata* 30CH	0.137±0.007	90.91
Laurocerasus 6CH + *Laurocerasus* 6CH + *Holarrhena antidysenterica* 30CH	0.161±0.017	89.32
Barosma crenata 30CH + *Holarrhena antidysenterica* 30CH + *Laurocerasus* 6CH	0.179±0.003	88.13
Doripenem	0.231±0.022	86.72

DISCUSSION-

Pseudomonas aeruginosa is ubiquitous[21]. Additionally, to the normal environmental condition for its growth, it causes hospital infections.

Pseudomonas aeruginosa is resistant to antibiotics. And once the bacteria turn out to be resistant to anti-biotic, they are not killed but on the contrary, they even grow and multiply even when under the influence of antibiotics[22]. Doripenem is used as a broad-spectrum antibiotic from Carbapenem group for chemical control. Antibiotic resistance is one of the major concerns because of the susceptibility of the patient to fall easily for such infection-causing bacterium and intrinsic resistance is given by the bacterium [23]. According to our survey, experimental studies of homoeopathic medicines against *Pseudomonas aeruginosa* is less recorded. These experiments were performed to see the antibacterial effect of homoeopathic medicines against *Pseudomonas aeruginosa* by performing the *in-vitro* experimental study. As per our experimental findings, there is significant inhibition of bacterium after administration of homoeopathic medicines.

The present study plan was to go for the well diffusion method using 96 microwell plates, which gave good results against the bacterium. The main highlight of the experiment was the homoeopathic medicines were used in conjunction. Posology[24][25] plays an important rule while prescribing to patients, so the doses were administered at different time intervals, which admittedly gave more inhibition than of Doripenem. The system of Homoeopathy works on the principle of dynamization[26]i.e. by boosting the immunity (vital force)[27] of the individual to fight against the infection, further, this experimental data can be used as the base for clinical trials. The clinical trial will be very helpful for the patients suffering because of infections caused by *Pseudomonas aeruginosa*.

Among the homoeopathic medicines used, all the medicines showed inhibition percentage above 70% when administered in single-dose, whereas *Barosma Crenata* 30CH, *Holarrhena Antidysenterica* 30CH and *Laurocerasus* 6CH gave maximum inhibition percentage i.e., 77.04%, 77.72% and 77.98% respectively. Hence these medicines with maximum inhibition percentage were used twice in 8 hours interval, which gave more per cent inhibition of the organism as compared to a single-dose administration. When the same medicines were administered in triple doses of 45µl per dose in 4 hours interval gave inhibition of more than 87%. Among all the medicines Laurocerasus 6CH gave most per cent inhibition as compared to all the medicines.

The antagonistic effects observed with homoeopathic medicines is less than Doripenem (90.78%), but Doripenem has been recorded showing resistance against some strains of *Pseudomonas aeruginosal*[28], with a lot of adverse drug reaction to the individual[29]. Whereas homoeopathy shows no adverse drug reaction, no side effects to the individual. In future, there is scope to further proceed with new ideas for this experiment.

Homoeopathic system of medicine is nowadays proving their effect based on evidence. To know about the anti-bacterial activity of homoeopathic medicines - *Barosma crenata*, *Holarrhena antidysenterica* and *Laurocerasus* on *Pseudomonas aeruginosa*, the study was conducted to support homoeopathy as evidence-based medicine. All the homoeopathic medicines selected for the study showed more or less anti-bacterial activity against *Pseudomonas*

aeruginosa. For further clinical trials, the pharmacognostic study is needed to understand the mode of action of selected medicines.

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